REMARKS

Applicant intends this response to be a complete response to the Examiner's **28 April 2006** Non-Final Office Action. Applicant has labeled the paragraphs in his response to correspond to the paragraph labeling in the Office Action for the convenience of the Examiner.

Withdrawal of Notice of Allowance

Applicant is advised that the Notice of Allowance mailed 2/10/06 is vacated.

The Examiner contends:

> If the issue fee has already been paid, applicant may request a refund or request that the fee be credited to a deposit account. However, applicant may wait until the application is either found allowable or held abandoned. If allowed, upon receipt of a new Notice of Allowance, applicant may request that the previously submitted issue fee be applied. If abandoned, applicant may request refund or credit to a specified Deposit Account. Prosecution on the merits of this application is reopened on claims 10, 13-19, and 50-56 in view of the new rejections set forth below.

Applicants are disappointed with this decision as the PCT versions of these newly cited references were made available to the Examiner as early as 2-13-2002. Although they were PCT applications,, they are identical to the new cited references and include claims of priority to the appropriate provisional applications. Applicants could have addressed these issues several years ago.

Claim Rejections - 35 USC § 102

Claims 10, 13-18, and 50-55 are rejected under 35 U.S.C. 102(e) as being anticipated by Korlach et al. (US 2006/0078937 Al). Applicants have added new claims 57-99 that include different compositional claims designed to distinguish over Korlach et al. and Schneider et al.

The Examiner contends:

> Korlach et al. disclose a composition comprising a polymerizing agent including a molecular tag covalently bonded to a site on the polymerizing agent and a monomer including a molecular tag, where at least one of the tags has a fluorescence property that undergoes a change before, during and/or after each of a sequence of monomer incorporations due to an interaction between the polymerizing agent tag and the monomer tag (claim 62), as stated in instant claim 10. Korlach et al. disclose a composition wherein the polymerizing agent is a polymerase or reverse transcriptase (claim 63), as stated in instant claim 13. Korlach et al. disclose a composition wherein the polymerase is selected from the group of Taq DNA polymerase, T7 DNA polymerase, Sequenase, and the Klenow fragment from E. coli

DNA polymerase (claim 64), as stated in instant claim 14. Korlach et al. disclose a composition wherein the reverse transcriptase comprises HIV reverse transcriptase (claim 65), as stated in instant claim 15. Korlach et al. disclose a composition wherein each of the monomers comprises a deoxynucleotide triphosphate (dNTP) and the monomer tag is covalently bonded to the 16 or 7 phosphate group of each dNTP (claim 66), as stated in instant claim 16. Korlach et al. disclose a composition wherein the tags comprise fluorescent tags, and the fluorescence property comprises an intensity, wavelength, and/or frequency of emitted fluorescent light (claim 67), as stated in instant claim 17. Korlach et al. disclose a composition wherein the fluorescence property is fluorescence resonance energy transfer (FRET) where either the monomer tag of the polymerase tag comprises a donor and the other tag comprises an acceptor and where FRET occurs when the two tags are in close proximity (claim 68), as stated in instant claim 18. Korlach et al. disclose a composition wherein the polymerase comprises *Taq* DNA Polymerase having a tag attached to an amino acid position at a specific amino acid of the *Taq* DNA polymerase that is less than 60Å from an incorporating nucleotide (claim 69). Korlach et al. disclose a composition comprising a polymerizing agent including a molecular tag covalently bonded to a site on the polymerizing agent and a deoxynucleotide triphosphate (dNTP) including a molecular tag covalently bonded to the β or γ phosphate group of the dNTP, where at least one of the tags has a fluorescence property that undergoes a change before, during and/or after each of a sequence of monomer incorporations due to an interaction between the polymerizing agent tag and the monomer (claim 70), as stated in instant claim 50. Korlach et al. disclose a composition wherein the polymerizing agent is a polymerase or reverse transcriptase (claim 71), as stated in instant claim 51. Korlach et al. disclose a composition wherein the polymerase is selected from the group consisting of Taq DNA polymerase, T7 DNA polymerase, Sequenase, and the Klenow fragment from E. coli DNA polymerase (claim 72), as stated in instant claim 52. Korlach et al. disclose a composition wherein the reverse transcriptase comprises HIV reverse transcriptase (claim 73), as stated in instant claim 53. Korlach et al. disclose a composition wherein the tags comprise fluorescent tags, and the fluorescence property comprises an intensity, wavelength, and/or frequency of emitted fluorescent light (claim 74), as stated in instant claim 54. Korlach et al. disclose a composition wherein the fluorescence property is fluorescence resonance energy transfer (FRET) where either the monomer tag of the polymerase tag comprises a donor and the other tag comprises an acceptor and where FRET occurs when the two tags are in close proximity (claim 75), as stated in instant claim 55. Korlach et al. disclose a composition wherein the polymerase comprises *Taq* DNA Polymerase having a tag attached to an amino acid position at a specific amino acid of the *Taq* DNA Polymerase, that is less than 60 A from an incorporating nucleotide (claim 76). Korlach et al. anticipate the limitations in claims 10, 13-18, and 50-55.

The prior art 6,982,146 B1 (Schneider et al.) is made of record and not relied upon; however, it is considered pertinent to applicant's disclosure.

Antedate Korlach et al.

Applicants **formally antedate** Korlach et al. as it pertains to the use of beta (β) phosphate, gamma (γ) phosphate and/or terminal phosphate labeled dNTPsin single molecule sequencing of a nucleic acids , and, respectfully request withdrawal of this rejection as it relates to claims 50-55, 57-78 and 89-99. The present invention was made before May 17, 2000. Applicant's attorney can attest via documentary evidence that the present invention was invented prior to May 17, 2000, which is the date of the filing of United States Patent Application Serial No. 09/572530 to Korlach et al.

The Korlach et al May 19, 1999 Provisional Does Not Disclose beta (β) and/or gamma (γ) phosphate labeled dNTPs. The May 17, 2000 filing date of the Korlach et al. upgrade application is the earliest date for which Korlach et al. can claim right to beta (β) and/or gamma (γ) phosphate labeled dNTPs.

Claims 50-55, 57-78 and 89-99 Are Free of Korlach et al.

Because Korlach et al. can only support disclosure of beta (β) phosphate and/or gamma (γ) phosphate labeled dNTPs as of May 17, 2000, and Applicants antedate this reference due to prior invention, Korlach et al. cannot anticipate claims 50-55, 57-78 and 89-99 of this application. Applicants also point out that neither Korlach et al. application discloses terminal phosphate labeled nucleotides - nucleotides that may include more the three phosphates. Applicants, therefore, respectfully request withdrawal of this section 102(e) rejection as it relates to claims 50-55, 57-78 and 89-99. Newly added claims 57-78 and 89-99 are fully supported in the specification and these claims do not add new matter.

No Korlach et al Application Disclose FRET using β and/or γ phosphate labeled dNTPs

The May 17, 2000 Korlach et al application does not even support FRET using β and/or γ phosphate labeled dNTPs. Even though United States Patent Application Serial No. 09/572530 filed May 17, 2000 to Korlach et al. disclosed beta (β) and/or gamma (γ) phosphate labeled dNTPs, **it did not include any disclosure, teaching or suggestion to use beta (β) and/or gamma (γ) phosphate labeled dNTPs in the context of FRET detection of incorporation events.** Moreover, the only teaching of Korlach et al. in the context of FRET lamented the problem associated with FRET signals from incorporated labeled nucleotide for up to 20 bases away from the current incorporation site or until the labeled nucleotides in the growing DNA chain moved more than 60Å away from the active site. 60Å is a generally agreed upon distance to support FRET. Korlach et al.

did disclose that this problem could be eliminated by photobleaching or photocleavage of the labels incorporated nucleotide prior to the next dNTP incorporation. Thus, the Korlach et al. FRET disclosure is only consistent with labeled dNTPs where the label persists in the developing primer DNA chain. In no way can one skilled in the art interpret these statements as disclosing or even suggesting the use of beta (β) and/or gamma (γ) phosphate labeled dNTPs in a FRET detection system. Furthermore, **there is not even a suggestion of the fact that by using beta (β) and/or gamma (γ) phosphate labeled dNTPs, one would not have to be concerned with continued FRET signals from previously incorporated nucleotides as none of them would have labels that could undergo FRET with the donor on the polymerase.**

US 6982146 Cannot Anticipate or Render Obvious Claim 50-55, 57-78 and 89-99

Applicants also point out that claims 50-55, 57-78 and 89-99 are not anticipated or rendered obvious by US 6,982,146 B1 (Schneider et al.), because that patent does not disclose, teach or even suggest labeling the dNTP at the beta and/or gamma phosphate or terminal phosphate. In fact, the absence of this teaching speaks volumes for the non-obviousness of use of beta and/or gamma phosphate or terminal phosphate labeled dNTPs in single molecular sequencing.

Obviousness of Claims 50-55, 57-78 and 89-99 over the 1999 Korlach et al Provisional Appl'n

Although Applicants have formally antedated United States Patent Application Serial No. 09/572530 to Korlach et al. as it pertains to beta (β), gamma (γ) and terminal phosphate labeled dNTPs - **United States Provisional Patent Application Serial No. 60/134827 to Korlach et al. filed May 19, 1999 contains no disclosure to beta (β), gamma (γ) or terminal phosphate labeled dNTPs**, Applicants take this opportunity to show that the 1999 Korlach et al. Provisional Application does not render claims 50-55, 57-78 and 89-99 obvious.

The 1999 Korlach et al. provisional application relates to sequencing using labeled dNTPs in sequencing, where the labels persist in the growing DNA chain, herein referred to as "persistent labels." In fact, the only disclosure relating to FRET clearly relates to only to dNTPs labeled with persistent labels. These persistent labels will contribute to the FRET signal until they pass out of an active FRET range or are photobleached or photocleaved in order to kill their FRET emissions. In this context, the Examiner has misunderstood the teaching of Korlach et al. as it related to FRET and the 60Å limitation. Korlach et al. states as follows:

> [0104] Detection of fluorescence resonance energy transfer (FRET) from a donor fluorophore (e.g., a donor attached to the polymerase) to adjacent nucleotide analog acceptors that are incorporated into the growing nucleic acid strand suggests a further elegant possibility of lowering background from incorporated nucleotides. **FRET only reaches very short distances including about 20 nucleotides and decays at the reciprocal sixth power of distance.** The excited donor molecule transfers its energy only to nearby acceptor fluorophores, which emit the spectrally resolved acceptor fluorescence of each labelled nucleotide as it is added. **Already incorporated nucleotides farther away from the donor would not contribute to the fluorescent signal since distance and orientation constraints of energy transfer reduce the effective range of observation to less than 60Å, thereby effectively eliminating background fluorescence from unincorporated nucleotides.** ***Without photobleaching, the method requires high sensitivity since repeat nucleotides leave the range of FRET at the same rate that new nucleotides are added, possibly creating sequence recognition ambiguity. Photobleaching or photochemical cleavage, or their combination as discussed above could resolve the problem.*** Photobleaching of the donor molecules using FRET can be avoided if it is the template nucleic acid that is attached and the donor bearing nucleic acid polymerizing enzyme is periodically replaced.

Korlach et al at paragraph 104 (emphasis added).

This language clearly does not support the claims asserted by Korlach et al.:

> wherein the polymerase comprises *Taq* DNA Polymerase having a tag attached to an amino acid position at a specific amino acid of the *Taq* DNA Polymerase, that is less than 60Å from an incorporating nucleotide.

Korlach et al at paragraph 104.

There is simply no way to twist this 60Å teaching into a teaching on the positioning of the donor fluorophore on the polymerase relative to the incorporating dNTP. The paragraph relates clearly and specifically only to how fast the persistent labels on the incorporated nucleotides will no longer contribute to the FRET signal. This interpretation is directly supported by the statement in paragraph 104 of Korlach et al. that:

> Without photobleaching, the method requires high sensitivity since repeat nucleotides leave the range of FRET at the same rate that new nucleotides are added, possibly creating sequence recognition ambiguity.

Korlach et al at paragraph 104.

Thus, it is clear that Korlach et al. teach absolutely nothing about the specific position of the label on the polymerase – the 60Å reference relates only to how far down the growing chain a persistent label will interact with a donor regardless of where the donor happens to be. In fact, for many polymerases, one cannot put a label 60Å from the active site as the polymerase is less is about

60-100Å in diameter.

One can claim that an ordinary artisan would know that this 60Å reference in Korlach et al related to the placement of the donor on the polymerase, but we reject this out of hand. Applicants, in the present application, went to great length to teach that the label cannot just be willy nilly placed on the polymerase or on molecules associated therewith. Applicants point out that the tag must be properly located on the polymerase. Korlach et al give no guidance on how one would go about labeling a polymerase. There are sites on the polymerase that cannot be labeled as it interferes with polymerase folding and/or polymerase activity. There are cites that are effectively shielded from the active site. With no teaching of how to tag a polymerase, one is left with the prospect of having to run hundreds and hundreds of reactions and mutations to find polymerases that would work. Disclosure that requires undue experimentation is non-enabling. The present application teaches clearly how and where to label. In fact, the application give exacts locations on one polymerase where cysteine substitution is made and the label attached. Korlach et al give the ordinary artisan absolutely no disclosure on labeling a polymerase.

Moreover, it is also apparent that Korlach et al. teach or suggest absolutely nothing about the use of beta (β) and/or gamma (γ) phosphate or terminal phosphate labeled dNTPs, especially in the context of FRET with a donor tagged polymerase. In fact, they teach fundamentally away from the use of such non-persistent dNTP tags. As the Federal Circuit has made it clear in the context of equivalents, infringement will not lie if an equivalent was forseeable at the time of filing - a doctrine akin to obviousness. The use of beta (β) and/or gamma (γ) phosphate or terminal phosphate labeled dNTPs in DNA sequencing with donor labeled polymerases was clearly not obvious to Korlach et al in 1999 – no disclosure of beta or gamma labeled dNTPs at all – or even in 2000 – no disclosure of beta and gamma labeled dNTPS used in combination with a donor labeled polymerase in a FRET context. This non-obviousness conclusion is further supported by US 6,982,146 B1 (Schneider et al.), where Schneider et al. disclosed in great detail a sequencing technology based solely on dNTPs labeled on the base, sugar or alpha phosphate – persistent labels. All of these labels, like the labels disclosed in the Korlach et al. provisional are not beta (β) and/or gamma (γ) phosphate or terminal phosphate labeled dNTPs. The idea of using beta (β) and/or gamma (γ) phosphate or terminal phosphate labeled dNTPs with a tagged polymerase, where dNTP incorporation is monitored by changes is a detectable property of the beta (β) and gamma (γ) phosphate labeled dNTPs and/or a label on the polymerase, including FRET, is disclosed only in the present application.

Claims 10 and 13-18

Turning our attention to claims 10 and 13-18, Applicants has amended claims 10 and 13-18, where appropriate, to require that the polymerase lacks 3' to 5' exonuclease activity. Support for the polymerizing agent lacking 3' to 5' exonuclease activity can be found at least on pages 40-41, 58 and 72-73. Nothing in either the 1999 or 2000 Korlach et al. application discloses that the polymerase lack 3' to 5' exonuclease activity.

Because Korlach et al. does not disclose polymerases lacking 3' to 5' exonuclease activity, Korlach et al. cannot anticipate claims 10, 13-18. Moreover, because there is not even any teaching or even a suggestion in Korlach et al. regarding polymerases lacking exonuclease activity, Korlach et al. cannot render claims 10, 13-18 obvious.

Schneider et al also does not disclose polymerases lacking 3' to 5' exonuclease activity. In fact, Schneider et al states:

> Polymerase: The enzyme which catalyzes the elongation of the primer strand, in the 5' to 3' direction along the nucleic acid template to be sequenced. Examples of polymerases which may be used in the method disclosed herein include, but are not limited to: the *E. coli* DNA polymerase I, specifically the Klenow fragment which has 3' to 5' exonuclease activity, *Taq* polymerase, reverse transcriptase, *E. coli* RNA polymerase, and wheat germ RNA polymerase 11.

Schneider et al at Col. 13, 19-26.

Thus, Schneider et al teaches straight away from polymerases lacking 3' to 5' exonuclease activity and cannot anticipate or render obvious claims 10, 13-18.

Claims 79-88

Korlach et al or Schneider et al also do not render new claims 79-88 obvious. There is no teaching in Korlach et al or Schneider et al concerning polymerizing agent lacking 3' to 5' exonuclease activity or the proper placement of the tag on the polymerizing agent should be ≤ 25Å from the incorporating monomers or that the site be located on a site that are not important to the proper functioning of the polymerizing agent. This limitation finds full support in the specification at pages 45-52, where Applicants go to considerable length to inform the ordinary skilled artisan how to select sites that do not kill the functioning of the polymerizing agent regardless of the manner of the killing the functioning of the polymerizing agent. The distance limitation can be found at the paragraphs bridging page 20 and 21.

Because nothing in Korlach et al speak to the proper placement of a tag on the polymerase, Korlach et al cannot anticipate or render obvious new claims 79-88.

Allowable Subject Matter

Claims 19 and 56 are objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims. Claims 19 and 26 recite specific amino acid positions of SEQ ID NO: 11 from *Taq* DNA polymerase I for attachment to a tag which are not disclosed in the prior art.

Applicants note that the application has allowable subject matter.

The Commissioner of Patents is authorized to debit any necessary fees or to credit any overpayments or refunds to Deposit Account No. 501518.

Having fully responded to the Examiner's Final Office Action, Applicant respectfully urges that is application be passed onto allowance.

If the Examiner requires additional information, then Applicants request that the Examiner contact their Attorney, Robert W. Strozier, at 713-977-7000.

Date: **7 June 2006**

Respectfully submitted,

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